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(54) Title: A METHOD OF INCREASING THE SPECIFICITY OF OXY-LNA OLIGONUCLEOTIDES

(57) Abstract: The present invention relates to the field of chimeric oligonucleotides composed of oxy-LNA monomers and at least one non-oxy-LNA monomer located within the distance of no more than three bases form or at the mismatch position(s) which are useful for the discrimination between fully matched target nucleic acids and target nucleic acids containing one or a few mismatches. This is achieved by modulating the ability of an oxy-LNA oligo to discriminate between its complementary nucleic acid target sequences and target sequences that comprise these mismatches, by incorporating at least one non-oxy-LNA monomer in the oxy-LNA oligo. In addition to the ability to modulate characteristics such as affinity, overall hydrophobicity and charge etc., non-oxy-LNAs can also be used to modulate the ability of "oxy-LNA" oligonucleotides to discriminate between matched and mismatched nucleic acid target sequences.

A method of increasing the specificity of oxy-LNA oligonucleotides

Field of Invention

The present invention relates to the field of chimeric oligonucleotides which are useful for the discrimination between fully matched target nucleic acids and target nucleic acids containing one or a few mismatches. The oligonucleotides used within the present invention are composed of oxy-LNA monomers and at least one non-oxy-LNA monomer located close to or at the mismatch position(s).

Background of the invention

Synthetic oligonucleotides are widely used compounds in disparate fields such as molecular biology and DNA-based diagnostics and therapeutics.

Therapeutics

In therapeutics, *e.g.*, oligonucleotides have been used successfully to block translation in vivo of specific mRNAs thereby preventing the synthesis of proteins which are undesired or harmful to the cell/organism. This concept of oligonucleotide mediated blocking of translation is known as the "antisense" approach. Mechanistically, the hybridising oligonucleotide is thought to elicit its effect by either creating a physical block to the translation process or by recruiting cellular enzymes that specifically degrades the mRNA part of the duplex (RNase H).

More recently, oligoribonucleotides and oligodeoxyribonucleotides and analogues thereof which combine RNase catalytic activity with the ability to sequence specifically interact with a complementary RNA target (ribozymes) have attracted much interest as antisense probes. Thus far ribozymes have been reported to be effective in cell cultures against both viral targets and oncogenes.

The use of oligonucleotides known as aptamers are also being actively investigated. This promising new class of therapeutic oligonucleotides are selected *in vitro* to specifically bind to a given target with high affinity, such as for example ligand receptors. Their binding characteristics are likely a reflection of the ability of oligonucleotides to form three dimensional structures held together by intramolecular nucleobase pairing.

Diagnostics

In molecular biology, oligonucleotides are routinely used for a variety of purposes such as for example (i) as hybridisation probes in the capture, identification and quantification of target nucleic acids (ii) as affinity probes in the purification of target nucleic acids (iii) as primers in sequencing reactions and target amplification processes such as the polymerase chain reaction (PCR) (iv) to clone and mutate nucleic acids and (vi) as building blocks in the assembly of macromolecular structures.

Diagnostics utilises many of the oligonucleotide based techniques mentioned above in particular those that lend themselves to easy automation and facilitate reproducible results with high sensitivity. The objective in this field is to use oligonucleotide based techniques as a means to, for example (i) tests humans, animals and food for the presence of pathogenic micro-organisms (ii) to test for genetic predisposition to a disease (iii) to identify inherited and acquired genetic disorders, (iv) to link biological deposits to suspects in crime trials and (v) to validate the presence of micro-organisms involved in the production of foods and beverages.

General considerations

To be useful in the extensive range of different applications outlined above, oligonucleotides have to satisfy a large number of different requirements. In antisense therapeutics, for instance, a useful oligonucleotide must be able to penetrate the cell membrane, have good resistance to extra- and intracellular nucleases and preferably have the ability to recruit endogenous enzymes like RNaseH. In DNA-based diagnostics and molecular biology other properties are important such as, *e.g.*, the ability of oligonucleotides to act as efficient substrates for a wide range of different enzymes evolved to act on natural nucleic acids, such as *e.g.* polymerases, kinases, ligases and phosphatases.

The fundamental property of oligonucleotides, however, which underlies all uses is their ability to recognise and hybridise sequence specifically to complementary single stranded nucleic acids employing either Watson-Crick hydrogen bonding (A-T and G-C) or other hydrogen bonding schemes such as the Hoogsteen mode.

There are two important terms that characterise the hybridisation properties of a given oligonucleotide: **affinity** and **specificity**

Affinity is a measure of the binding strength of the oligonucleotide to its complementary target sequence (expressed as the thermostability (T_m) of the duplex). Each nucleobase pair in the duplex adds to the thermostability and thus affinity increases with increasing size (no. of nucleobases) of the oligonucleotide.

Nucleobases are aromatic molecules. When two complementary strands of nucleic acids hybridize to form a double helix the nucleobases forms hydrogen bonds between the two strands and also interacts hydrophobically with adjacent nucleobases. This latter phenomenon is known as stacking and adds favorably to the overall stability of the helix.

Specificity is a measure of the ability of the oligonucleotide to discriminate between a fully complementary and a mismatched target sequence. In other words, specificity is a measure of the loss of affinity associated with mismatched nucleobase pairs in the target. At constant oligonucleotide size the specificity increases with increasing number of mismatches between the oligonucleotide and its targets (*i.e.* the percentage of mismatches increases). Conversely, specificity decreases when the size of the oligonucleotide is increased at a constant number of mismatches (*i.e.* the percentage of mismatches decreases). Stated another way, an increase in the affinity of an oligonucleotide occurs at the expense of specificity and vice-versa.

This property of oligonucleotides creates a number of problems for their practical use. In lengthy diagnostic procedures, for instance, the oligonucleotide needs to have both high affinity to secure adequate sensitivity of the test and high specificity to avoid false positive results. Likewise, an oligonucleotide used as antisense probes needs to have both high affinity for its target mRNA to efficiently impair its translation and high specificity to avoid the unintentional blocking of the expression of other proteins. With enzymatic reactions, like, *e.g.*, PCR amplification, the affinity of the oligonucleotide primer must be high enough for the primer/target duplex to be stable in the temperature range where the enzymes exhibits activity, and specificity needs to be high enough to ensure that only the correct target sequence is amplified.

Given the shortcomings of natural oligonucleotides, new approaches for enhancing specificity and affinity would be highly useful for DNA-based therapeutics, diagnostics and for molecular biology techniques in general.

Locked Nucleic Acid (LNA) is a novel, nucleic bicyclic acid analogue in which the 2'- and 4' position of the furanose ring are linked by an O-methylene (oxy-LNA), S-methylene (thio-LNA) or NH_2-methylene moiety (amino-LNA). This linkage restricts the conformational freedom of the furanose ring and leads to an increase in affinity which is by far the highest ever reported for a DNA analogue (WO 99/14226).

Given this very high affinity, LNA makes possible the design of short oligonucleotides which maintain a useful high affinity for their complementary target nucleic acids whilst providing a much improved specificity as compared to oligonucleotides composed of standard DNA, RNA or other moderate affinity DNA analogues.

Detailed description of the invention.

In many applications it will be desirable to use oligonucleotides containing both "non-oxy LNA monomers" and "oxy-LNA" monomers. For instance when used as antisense compounds it may be advantageous to increase the overall hydrophobicity of the oxy-LNA oligonucleotide by incorporating non-oxy-LNA monomers such as thio-LNA monomers thereby potentially increasing the ability of the oligonucleotide to pass the cell membrane. Likewise, non oxy-LNA monomers such as amino-LNA may be used to impose positive charge on the oxy-LNA oligonucleotide thereby changing its absorptive properties or modulating its pharmacokinetic properties.

Non-Oxy LNA monomers may also be used to decrease the overall affinity of an oxy-LNA oligonucleotide for its complementary nucleic acids. This can be achieved by replacing some of the oxy-LNA monomers in the oxy-LNA oligonucleotide by for instance lower affinity DNA or RNA monomers. Likewise, non-oxy-LNA monomers such as for instance thio-LNA and amino-LNA may be used to increase the relative affinity difference of an oxy-LNA oligonucleotide for its DNA and RNA complement.

We have now found that in addition to the ability to modulate characteristics such as affinity, overall hydrophobicity and charge etc., non-oxy-LNAs can also be used to modulate the ability of "oxy-LNA" oligonucleotides to discriminate between matched and mismatched nucleic acid target sequences. For instance, an oligo with a centre block of five oxy-LNAs surrounding a mismatch position were found to be better at discriminating between

its fully complementary and mismatched target than the corresponding oligo were the oxy-LNA on either side of the mismatch position were substituted by the corresponding DNA monomers. Likewise, the specificity of the oligonucleotide could be increased by substituting the oxy-LNA at the mismatch position, or the oxy-LNA monomers at either side of the mismatch position, with the corresponding thio-LNA monomers. Hence, the use of non-oxy-LNA monomers offers an attractive means for either decreasing the specificity of oxy-LNA oligonucleotides in cases were the oligonucleotide need to exhibit some mutation tolerance, such as for instance when used in the diagnosis of biological specimens which exhibit considerable sequence variation in their genome, or increasing the specificity in cases were the oligonucleotide needs to be able to stringently differentiate between closely related nucleic acids.

It is anticipated that the observed influence of non-oxy-LNAs on the specificity of oxy-LNA oligonucleotides may be due to many factors including steric effects, the ability of the nucleobases to interact by "stacking" or their ability to exclude water molecules from the helix.

The ability to modulate the specificity of "oxy-LNA" oligonucleotides as described above is of major importance in a wide variety of applications in molecular biology, DNA diagnostics and therapeutics and will be immediately apparent to one of ordinary skills in the art.

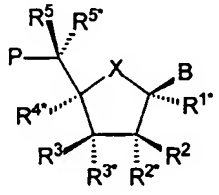
Accordingly, the present invention relates to the specific use of at least one "non-oxy-LNA" monomer in the design of an "oxy-LNA" oligo to modulate the ability of the oligo to discriminate between its complementary nucleic acid target sequence and target sequences containing one or more Watson-Crick mismatches. The "non-oxy-LNA" monomers may be i) used in place of an oxy-LNA monomer in the "oxy-LNA oligo" to be modified such that the overall size of the chimeric oligo is maintained (*i.e.* substitutions) or it may be ii) inserted into the "oxy-LNA" oligo to be modified such that the overall size of the chimeric oligo is increased (*i.e.* insertions) or used in any of the combinations of insertions and substitutions when more than one "non-oxy-LNA" monomer is used.

If the " non-oxy-LNA(s)" are used in place of an "oxy-LNA" monomer in the "oxy-LNA oligo" to be modified, the resulting chimeric oligo may have a decreased thermostability towards its complementary target nucleic acid as compared to the unmodified "oxy-LNA oligo", whereas if the " non-oxy-LNA" is inserted into the "oxy-LNA oligo" to be modified,

the resulting 1 base larger oligo may have a higher thermostability than the non-modified "oxy-LNA oligo". In a preferred embodiment, the modification of the "oxy-LNA oligo" by " non-oxy-LNA" monomer(s) is/are made in such a way that the resulting chimeric oligo displays a thermostability which is within ± 10°C, preferably ± 5°C of the thermostability of the non-modified "oxy-LNA oligo". If two or more non-oxy-LNAs are used to modify an "oxy-LNA oligo" these temperature limits can be maintained by the combined used of substitutions and insertions.

An "oxy-LNA oligo" is defined herein as an oligonucleotide containing at least 50% oxy-LNA monomers and one or more monomers which may be any of a multitude of different "non-oxy-LNA".

An "oxy-LNA monomer" is defined herein as a nucleotide monomer of the formula Ia



Ia

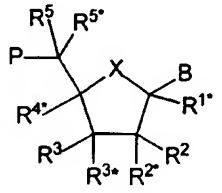
wherein X is oxygen; B is a nucleobase; R^{1*}, R^2, R^3, R^5 and R^{5*} are hydrogen; P designates the radical position for an internucleoside linkage to a succeeding monomer, or a 5'-terminal group, R^{3*} is an internucleoside linkage to a preceding monomer, or a 3'-terminal group; and R^{2*} and R^{4*} together designate -O-CH_2- where the oxygen is attached in the 2'-position.

The term "nucleobase" covers the naturally occurring nucleobases adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U) as well as non-naturally occuring nucleobases such as xanthine, diaminopurine, 8-oxo-N^6-methyladenine, 7-deazaxanthine, 7-deazaguanine, N^4,N^4-ethanocytosin, N^6,N^6-ethano-2,6-diaminopurine, 5-methylcytosine, 5-(C^3-C^6)-alkynylcytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridin, isocytosine, isoguanin, inosine and the "non-naturally occurring" nucleobases described in Benner et al., U.S. Pat No. 5,432,272 and Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol. 25, pp 4429-4443. The term "nucleobase" thus includes not only the known purine and pyrimidine heterocycles, but also

heterocyclic analogues and tautomers thereof. It should be clear to the person skilled in the art that various nucleobases which previously have been considered "non-naturally occurring" have subsequently been found in nature.

A "non-oxy-LNA" monomer is broadly defined as any nucleoside (i.e. a glycoside of a heterocyclic base) which is not itself an oxy-LNA but which can be used in combination with oxy-LNA monomers to construct oligos which have the ability to bind sequence specifically to complementary nucleic acids. Examples of non-oxy-LNA monomers include 2'-deoxynucleotides (DNA) or nucleotides (RNA) or any analogues of these monomers which are not oxy-LNA, such as for example the thio-LNA and amino-LNA described by Wengel and coworkers (Singh et al. J. Org. Chem. 1998, 6, 6078-9, and the derivatives described in Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol 25, pp 4429-4443.

As mentioned above, a wide variety of modifications of the deoxynucleotide skeleton can be contemplated and one large group of possible non-oxy-LNA can be described by the following formula I



I

wherein X is -O-; B is selected from nucleobases; R^{1*} is hydrogen;

P designates the radical position for an internucleoside linkage to a succeeding monomer, or a 5'-terminal group, such internucleoside linkage or 5'-terminal group optionally including the substituent R^5, R^5 being hydrogen or included in an internucleoside linkage,

R^{3*} is a group P* which designates an internucleoside linkage to a preceding monomer, or a 3'-terminal group;

one or two pairs of non-geminal substituents selected from the present substituents of R^2, R^{2*}, R^3, R^{4*}, may designate a biradical consisting of 1-4 groups/atoms selected from $-C(R^aR^b)-$, $-C(R^a)=C(R^a)-$, $-C(R^a)=N-$, $-O-$, $-S-$, $-SO_2-$, $-N(R^a)-$, and $>C=Z$,

wherein Z is selected from -O-, -S-, and $-N(R^a)-$, and R^a and R^b each is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands,

said possible pair of non-geminal substituents thereby forming a monocyclic entity together with (i) the atoms to which said non-geminal substituents are bound and (ii) any intervening atoms; and

each of the substituents R^2, R^{2*}, R^3, R^{4*} which are present and not involved in the possible biradical is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands;

and basic salts and acid addition salts thereof;

with the proviso the monomer is not oxy-LNA.

Particularly preferred non-oxy-LNA monomers are 2'-deoxyribonucleotides, ribonucleotides, and analogues thereof that are modified at the 2'-position in the ribose, such as 2´-O-methyl, 2´-fluoro, 2´-trifluoromethyl, 2´-O-(2-methoxyethyl), 2´-O-aminopropyl, 2´-O-dimethylamino-oxyethyl, 2´-O-fluoroethyl or 2´-O-propenyl, and analogues wherein the modification involves both the 2´and 3' position, preferably such analogues wherein the

modifications links the 2'- and 3'-position in the ribose, such as those described by Wengel and coworkers (Nielsen et al., J. Chem. Soc., Perkin Trans. 1, 1997, 3423-33, and in WO 99/14226), and analogues wherein the modification involves both the 2'- and 4'-position, preferably such analogues wherein the modifications links the 2'- and 4'-position in the ribose, such as analogues having a $-CH_2-S-$ or a $-CH_2-NH-$ or a $-CH_2-NMe-$ bridge (see Wengel and coworkers in Singh et al. J. Org. Chem. 1998, 6, 6078-9). Although non-oxy-LNA monomers having the β-D-ribo configuration are often the most applicable, further interesting examples (and in fact also applicable) of non-oxy-LNA are the stereoisomeric of the natural β-D-ribo configuation. Particularly interesting are the α-L-ribo, the β-D-xylo and the α-L-xylo configurations (see Beier et al., Science, 1999, 283, 699 and Eschenmoser, Science, 1999, 284, 2118), in particular those having a 2'-4' $-CH_2-S-$, $-CH_2-NH-$, $-CH_2-O-$ or $-CH_2-NMe-$ bridge (see Wengel and coworkers in Rajwanshi et al., Chem. Commun., 1999, 1395 and Rajwanshi et al., Chem. Commun., 1999, submitted)

In the present context, the term "oligonucleotide" which is the same as "oligomer" which is the same as "oligo" means a successive chain of nucleoside monomers (*i.e.* glycosides of heterocyclic bases) connected via internucleoside linkages. The linkage between two successive monomers in the oligo consist of 2 to 4, preferably 3, groups/atoms selected from $-CH_2-$, $-O-$, $-S-$, $-NR^H-$, $>C=O$, $>C=NR^H$, $>C=S$, $-Si(R'')_2-$, $-SO-$, $-S(O)_2-$, $-P(O)_2-$, $-PO(BH_3)-$, $-P(O,S)-$, $-P(S)_2-$, $-PO(R'')-$, $-PO(OCH_3)-$, and $-PO(NHR^H)-$, where R^H is selected from hydrogen and C_{1-4}-alkyl, and R'' is selected from C_{1-6}-alkyl and phenyl. Illustrative examples of such linkages are $-CH_2-CH_2-CH_2-$, $-CH_2-CO-CH_2-$, $-CH_2-CHOH-CH_2-$, $-O-CH_2-O-$, $-O-CH_2-CH_2-$, $-O-CH_2-CH=$ (including R^5 when used as a linkage to a succeeding monomer), $-CH_2-CH_2-O-$, $-NR^H-CH_2-CH_2-$, $-CH_2-CH_2-NR^H-$, $-CH_2-NR^H-CH_2-$, $-O-CH_2-CH_2-NR^H-$, $-NR^H-CO-O-$, $-NR^H-CO-NR^H-$, $-NR^H-CS-NR^H-$, $-NR^H-C(=NR^H)-NR^H-$, $-NR^H-CO-CH_2-NR^H-$, $-O-CO-O-$, $-O-CO-CH_2-O-$, $-O-CH_2-CO-O-$, $-CH_2-CO-NR^H-$, $-O-CO-NR^H-$, $-NR^H-CO-CH_2-$, $-O-CH_2-CO-NR^H-$, $-O-CH_2-CH_2-NR^H-$, $-CH=N-O-$, $-CH_2-NR^H-O-$, $-CH_2-O-N=$ (including R^5 when used as a linkage to a succeeding monomer), $-CH_2-O-NR^H-$, $-CO-NR^H-CH_2-$, $-CH_2-NR^H-O-$, $-CH_2-NR^H-CO-$, $-O-NR^H-CH_2-$, $-O-NR^H-$, $-O-CH_2-S-$, $-S-CH_2-O-$, $-CH_2-CH_2-S-$, $-O-CH_2-CH_2-S-$, $-S-CH_2-CH=$ (including R^5 when used as a linkage to a succeeding monomer), $-S-CH_2-CH_2-$, $-S-CH_2-CH_2-O-$, $-S-CH_2-CH_2-S-$, $-CH_2-S-CH_2-$, $-CH_2-SO-CH_2-$, $-CH_2-SO_2-CH_2-$, $-O-SO-O-$, $-O-S(O)_2-O-$, $-O-S(O)_2-CH_2-$, $-O-S(O)_2-NR^H-$, $-NR^H-S(O)_2-CH_2-$, $-O-S(O)_2-CH_2-$, $-O-P(O)_2-O-$, $-O-P(O,S)-O-$, $-O-P(S)_2-O-$, $-S-P(O)_2-O-$, $-S-P(O,S)-O-$, $-S-P(S)_2-O-$, $-O-P(O)_2-S-$, $-O-P(O,S)-S-$, $-O-P(S)_2-S-$, $-S-P(O)_2-S-$, $-S-P(O,S)-S-$, $-S-P(S)_2-S-$, $-O-PO(R'')-O-$, $-O-PO(OCH_3)-O-$, $-O-PO-$

(OCH_2CH_3)-O-, -O-PO(OCH_2CH_2S-R)-O-, -O-PO(BH_3)-O-, -O-PO(NHR^N)-O-, -O-P(O)$_2$-NR^H-, -NR^H-P(O)$_2$-O-, -O-P(O,NR^H)-O-, -CH_2-P(O)$_2$-O-, -O-P(O)$_2$-CH_2-, and -O-Si(R")$_2$-O-; among which -CH_2-CO-NR^H-, -CH_2-NR^H-O-, -S-CH_2-O-, -O-P(O)$_2$-O-, -O-P(O,S)-O-, -O-P(S)$_2$-O-, -NR^H-P(O)$_2$-O-, -O-P(O,NR^H)-O-, -O-PO(R")-O-, -O-PO(CH_3)-O-, and -O-PO(NHR^N)-O-, where R^H is selected form hydrogen and C_{1-4}-alkyl, and R" is selected from C_{1-6}-alkyl and phenyl, are especially preferred. Further illustrative examples are given in Mesmaeker et. al., Current Opinion in Structural Biology 1995, 5, 343-355 and Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol 25, pp 4429-4443. The left-hand side of the internucleoside linkage is bound to the 5-membered ring as substituent P* at the 3'-position, whereas the right-hand side is bound to the 5'-position of a preceding monomer.

The term "succeeding monomer" relates to the neighbouring monomer in the 5'-terminal direction and the "preceding monomer" relates to the neighbouring monomer in the 3'-terminal direction.

Monomers are referred to as being "complementary" if they contain nucleobases that can form hydrogen bonds according to Watson-Crick base-pairing rules (e.g. G with C, A with T or A with U) or other hydrogen bonding motifs such as for example diaminopurine with T, inosine with C, pseudoisocytosine with G, etc.

The presently disclosed chimeric oligos which display a specificity different from that of the parent oxy-LNA oligonucleotide contain at least 50% (fifty percent) (by numbers) of oxy-LNA, such as at least 55%, e.g. at least 60%, such as at least 65%, e.g. at least 70%, such as at least 75%, e.g. at least 80%, such as at least 85%, e.g. at least 90%, such as at least 95%, and the chimeric oligo is furthermore designed in such a way that at least one non-oxy-LNA monomer is located either at or within a distance of no more than three bases from the mismatch position(s), such as at a distance of two bases from the mismatch position, e.g. at a distance of one base from the mismatch position, e.g. at the mismatch position.

The physical location of the non-oxy-LNA monomer relative to the mismatch position(s) and the nature and number of the non-oxy-LNA monomer(s) are important determinants for how much the specificity will be increased or decreased in the resulting chimeric oligo as compared to the unmodified all oxy-LNA oligo. In general, the non-oxy-LNA monomers

should be located within 3 bases on either side of the mismatch to produce a measurable effect on the specificity of the oligonucleotide. When only 1 non-oxy-LNA is used, it may be located on either the 3'- or the 5' -side of the mismatch or at the mismatch position. When 2 non-oxy-LNA are used they may both be located either contiguous or non-contiguous on either the 5'- or the 3' side of the mismatch or more preferably they are located either symmetrically or asymmetrically on either side of the mismatch at a maximum of 3, more preferably 2 and even more preferably 1 base from the mismatch position. Equally preferably, 1 of the non-oxy-LNAs may be located at the mismatch position and the other may be located at either the 5'- or 3'-side of the mismatch position at a miximum distance of 3 bases, preferably 2 bases and most preferably 1 base from the mismatch position. When 3 or more non-oxy-LNAs are used they may all be located either contiguous or non-contiguous on either the 5'- or the 3' side of the mismatch or more preferably they are located with 2 contiguous or non-contiguous non-oxy-LNAs on one side of the mismacth and 1 non-oxy-LNA on the other side, and with a miximum distance of all non-oxy-LNAs of 3 bases from the mismatch position. When 3 or more non-oxy-LNAs are used 1 may also be located at the mismatch position and the others located either symmetrically or asymmetrically or contiguous or non-contiguous within a distance of no more than 3 bases on either side.

When a non-oxy LNA monomer is located at the mismatch position, it may be advantageous that it carries a modified heterocyclic base which exhibit increased binding affinity as compared to the unmodified heterocyclic base. The rationale for using such modified bases is that they expand the difference in thermostability between their matched and mismatched targets, *i.e.* the specificity of the oligo increases. Examples of higher affinity modified heterocyclic bases that can be used in place of the normal nucleobases are for instance 2,6-diamino-purine which, compared with the normal nucleobase adenine, allows an additional H-bond to be formed with the U and T nucleobase. Further illustrative examples of modified nucleobase that can be used to increase duplex stability include the 7-halo-7-deaza and the 7-propyne-7-deaza purines and others described in for instance Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol. 25, pp. 4429-4443.

When the modified oxy-LNA oligo contain at least two non-oxy-LNA monomers these may contain the same or different nucleobases at the 1'-position and be identical at all other

positions or they may contain the same or different nucleobases at the 1'-position and be non-identical at at least one other position.

The resulting oligo containing oxy-LNA monomers and non-oxy-LNA monomers can thus be characterized by the general formula

$$5'\text{-}X_{n1}Y_{m1}X_pY_{m2}X_{n2}\text{-}3'$$

wherein X is oxy-LNA and Y is non-oxy-LNA, n1 and n2 are integers from 0-50, m1 and m2 are integers from 0-5, such as from 0-4, e.g. from 0-3, e.g. from 0-2, such as from 0-1, p is an integer from 0-3, e.g. from 0-2, such as from 0-1 with the proviso that the sum of n1+m1+p+m2+n2 is in the range of 5-100, such as 8, e.g. 9, e.g. 10, e.g. 11, e.g. 12, e.g. 13, e.g. 14, e.g. 15, e.g. 16, e.g. 17, e.g. 18, e.g. 19, e.g. 20, e.g. 21, e.g. 22, e.g. 23, e.g. 24, e.g. 25, e.g. 26, e.g. 27, e.g. 28, e.g. 29, e.g. 30, e.g. 35, e.g. 40, e.g. 45, e.g. 50, e.g. 60, e.g. 70, e.g. 80, e.g. 90, such as 100.

The chimeric oligos of the present invention are highly suitable for a variety of diagnostic purposes such as for the isolation, purification, amplification, detection, identification, quantification, or capture of natural – such as DNA, mRNA or non-protein coding cellular RNAs, such as tRNA, rRNA, snRNA and scRNA – or synthetic nucleic acids, *in vivo* or *in vitro*.

The oligomer can comprise a photochemically active group, a thermochemically active group, a chelating group, a reporter group, or a ligand that facilitates the direct of indirect detection of the oligomer or the immobilisation of the oligomer onto a solid support. Such group are typically attached to the oligo when it is intended as a probe for *in situ* hybridisation, in Southern hydridisation, Dot blot hybridisation, reverse Dot blot hybridisation, or in Northern hybridisation.

When the photochemically active group, the thermochemically active group, the chelating group, the reporter group, or the ligand includes a spacer (K), said spacer may comprise a chemically cleavable group.

In the present context, the term "photochemically active groups" covers compounds which are able to undergo chemical reactions upon irradiation with light. Illustrative examples of

functional groups hereof are quinones, especially 6-methyl-1,4-naphtoquinone, anthraquinone, naphtoquinone, and 1,4-dimethyl-anthraquinone, diazirines, aromatic azides, benzophenones, psoralens, diazo compounds, and diazirino compounds.

In the present context "thermochemically reactive group" is defined as a functional group which is able to undergo thermochemically-induced covalent bond formation with other groups. Illustrative examples of functional parts thermochemically reactive groups are carboxylic acids, carboxylic acid esters such as activated esters, carboxylic acid halides such as acid fluorides, acid chlorides, acid bromide, and acid iodides, carboxylic acid azides, carboxylic acid hydrazides, sulfonic acids, sulfonic acid esters, sulfonic acid halides, semicarbazides, thiosemicarbazides, aldehydes, ketones, primary alkohols, secondary alcohols, tertiary alcohols, phenols, alkyl halides, thiols, disulphides, primary amines, secondary amines, tertiary amines, hydrazines, epoxides, maleimides, and boronic acid derivatives.

In the present context, the term "chelating group" means a molecule that contains more than one binding site and frequently binds to another molecule, atom or ion through more than one binding site at the same time. Examples of functional parts of chelating groups are iminodiacetic acid, nitrilotriacetic acid, ethylenediamine tetraacetic acid (EDTA), aminophosphonic acid, etc.

In the present context, the term "reporter group" means a group which is detectable either by itself or as a part of an detection series. Examples of functional parts of reporter groups are biotin, digoxigenin, fluorescent groups (groups which are able to absorb electromagnetic radiation, *e.g.* light or X-rays, of a certain wavelength, and which subsequently reemits the energy absorbed as radiation of longer wavelength; illustrative examples are dansyl (5-dimethylamino)-1-naphthalenesulfonyl), DOXYL (N-oxyl-4,4-dimethyloxazolidine), PROXYL (N-oxyl-2,2,5,5-tetramethylpyrrolidine), TEMPO (N-oxyl-2,2,6,6-tetramethylpiperidine), dinitrophenyl, acridines, coumarins, Cy3 and Cy5 (trademarks for Biological Detection Systems, Inc.), erytrosine, coumaric acid, umbelliferone, texas red, rhodamine, tetramethyl rhodamine, Rox, 7-nitrobenzo-2-oxa-1-diazole (NBD), pyrene, fluorescein, Europium, Ruthenium, Samarium, and other rare earth metals), radioisotopic labels, chemiluminescence labels (labels that are detectable via the emission of light during a chemical reaction), spin labels (a free radical (*e.g.* substituted organic nitroxides) or other paramagnetic probes (*e.g.* Cu^{2+}, Mg^{2+}) bound to a biological molecule being detect-

able by the use of electron spin resonance spectroscopy), enzymes (such as peroxidases, alkaline phosphatases, β-galactosidases, and glycose oxidases), antigens, antibodies, haptens (groups which are able to combine with an antibody, but which cannot initiate an immune response by itself, such as peptides and steroid hormones), carrier systems for cell membrane penetration such as: fatty acid residues, steroid moieties (cholesteryl), vitamin A, vitamin D, vitamin E, folic acid peptides for specific receptors, groups for mediating endocytose, epidermal growth factor (EGF), bradykinin, and platelet derived growth factor (PDGF). Especially interesting examples are biotin, fluorescein, Texas Red, rhodamine, dinitrophenyl, digoxigenin, Ruthenium, Europium, Cy5, Cy3, etc.

In the present context "ligand" means something which binds. Ligands can comprise functional groups such as: aromatic groups (such as benzene, pyridine, naphtalene, anthracene, and phenanthrene), heteroaromatic groups (such as thiophene, furan, tetrahydrofuran, pyridine, dioxane, and pyrimidine), carboxylic acids, carboxylic acid esters, carboxylic acid halides, carboxylic acid azides, carboxylic acid hydrazides, sulfonic acids, sulfonic acid esters, sulfonic acid halides, semicarbazides, thiosemicarbazides, aldehydes, ketones, primary alcohols, secondary alcohols, tertiary alcohols, phenols, alkyl halides, thiols, disulphides, primary amines, secondary amines, tertiary amines, hydrazines, epoxides, maleimides, C_1-C_{20} alkyl groups optionally interrupted or terminated with one or more heteroatoms such as oxygen atoms, nitrogen atoms, and/or sulphur atoms, optionally containing aromatic or mono/polyunsaturated hydrocarbons, polyoxyethylene such as polyethylene glycol, oligo/polyamides such as poly-β-alanine, polyglycine, polylysine, peptides, oligo/polysaccharides, oligo/polyphosphates, toxins, antibiotics, cell poisons, and steroids, and also "affinity ligands", *i.e.* functional groups or biomolecules that have a specific affinity for sites on particular proteins, antibodies, poly- and oligosaccharides, and other biomolecules.

It will be clear for the person skilled in the art that the above-mentioned specific examples under DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands correspond to the "active/functional" part of the groups in question. For the person skilled in the art it is furthermore clear that DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands are typically represented in the form M-K- where M is the "active/functional" part of the group in question and where K is a spacer through which the "active/functional" part is attached to the 5- or 6-membered ring. Thus, it should be

understood that the group B, in the case where B is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, has the form M-K-, where M is the "active/functional" part of the DNA intercalator, photochemically active group, thermochemically active group, chelating group, reporter group, and ligand, respectively, and where K is an optional spacer comprising 1-50 atoms, preferably 1-30 atoms, in particular 1-15 atoms, between the 5- or 6-membered ring and the "active/functional" part.

In the present context, the term "spacer" means a thermochemically and photochemically non-active distance-making group and is used to join two or more different moieties of the types defined above. Spacers are selected on the basis of a variety of characteristics including their hydrophobicity , hydrophilicity, molecular flexibility and length (*e.g.* see Hermanson et. al., "Immobilized Affinity Ligand Techniques", Academic Press, San Diego, California (1992), p. 137-ff). Generally, the length of the spacers are less than or about 400 Å, in some applications preferably less than 100 Å. The spacer, thus, comprises a chain of carbon atoms optionally interrupted or terminated with one or more heteroatoms, such as oxygen atoms, nitrogen atoms, and/or sulphur atoms. Thus, the spacer K may comprise one or more amide, ester, amino, ether, and/or thioether functionalities, and optionally aromatic or mono/polyunsaturated hydrocarbons, polyoxyethylene such as polyethylene glycol, oligo/polyamides such as poly-β-alanine, polyglycine, polylysine, and peptides in general, oligosaccharides, oligo/polyphosphates. Moreover the spacer may consist of combined units thereof. The length of the spacer may vary, taking into consideration the desired or necessary positioning and spatial orientation of the "active/functional" part of the group in question in relation to the 5- or 6-membered ring. In particularly interesting embodiments, the spacer includes a chemically cleavable group. Examples of such chemically cleavable groups include disulphide groups cleavable under reductive conditions, peptide fragments cleavable by peptidases, etc.

An attractive possibility of the invention is to use the chimeric oxy-LNA in the construction of high specificity oligo arrays wherein a multitude of different oligos are affixed to a solid surface in a predetermined pattern (Nature Genetics, suppl. vol. 21, Jan 1999, 1-60 and WO 96/31557). The usefulness of such an array, which can be used to simultaneously analyse a large number of target nucleic acids, depends to a large extend on the specificity of the individual oligos bound to the surface. The target nucleic acids may carry a de-

tectable label or be detected by incubation with suitable detection probes which may also be chimeric oxy-LNA oligos.

An additional object of the present invention is to provide oligos with combines an increased ability to discriminate between complementary and mismatched targets with the ability to act as substrates for nucleic acid active enzymes such as for example DNA and RNA polymerases, ligases, phosphatases. Such oligos may be used for instance as primers for sequencing nucleic acids and as primers in any of the several well known amplification reactions, such as the PCR reaction.

In a further aspect, the chimeric oligos are used to construct new affinity pairs with exhibit exquisite specificity towards each other. The affinity constants can easily be adjusted over a wide range and a vast number of affinity pairs can be designed and synthesised. One part of the affinity pair can be attached to the molecule of interest (*e.g.* proteins, amplicons, enzymes, polysaccharides, antibodies, haptens, peptides, etc.) by standard methods, while the other part of the affinity pair can be attached to *e.g.* a solid support such as beads, membranes, micro-titer plates, sticks, tubes, etc. The solid support may be chosen from a wide range of polymer materials such as for instance polypropylene, polystyrene, polycarbonate or polyethylene. The affinity pairs may be used in selective isolation, purification, capture and detection of a diversity of the target molecules.

Another aspect of the present invention is to exploit non-oxy-LNA monomers to design oligonucleotides which exhibit low specificity for their target nucleic acid sequences. Such oligonucleotides are particularly useful as probes in the purification, isolation and detection of for instance pathogenic organisms such as vira, bacteria and fungi etc. that exhibit considerable sequence variation in their genome, such as for instance the HIV and HCV virus. Also, low specificity probes are useful as generic tools for the purification, isolation, amplification and detection of nucleic acids from groups of related species such as for instance rRNA from gram-positive or gram negative bacteria, fungi, mammalian cells etc.

Yet another application of the present invention is the use of one or more chimeric oxy-LNA oligos as an aptamer in molecular diagnostics, e.g. in RNA mediated catalytic processes, in specific binding of antibiotics, drugs, amino acids, peptides, structural proteins, protein receptors, protein enzymes, saccharides, polysaccharides, biological cofactors,

nucleic acids, or triphosphates or in the separation of enantiomers from racemic mixtures by stereospecific binding.

A further application of the present invention is the use of chimeric oxy-LNA oligomers for labelling of cells, e.g. in methods wherein the label allows the cells to be separated from unlabelled cells.

Yet another application of the present invention is the use of one or more chimeric oxy-LNA oligos conjugated to a compound selected from proteins, amplicons, enzymes, polysaccharides, antibodies, haptens, and peptides.

Yet another application of the present invention is the use of one or more chimeric oxy-LNA oligos in a kit for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids, the kit comprising a reaction body and one or more oligomers of the invention. Additionally, the oligomers may be immobilised onto said reactions body.

A very important aspect of the present invention is to provide oligos which are suitable as either antisense, antigene or ribozyme therapeutic agents. In all these application, the ability to interact with exquisite specificity with only the correct target sequence is a vital determinant for both drug efficacy and safety.

Examples:

Non-oxy LNA monomers of the thio-LNA type can be used to increase the specificity of an oxy-LNA containing oligonucleotide.

The thermostability of several different oligonucleotides comprising both oxy and non-oxy-LNA monomers were determined spectrofotometrically using a spectrophotometer equipped with a thermoregulated Peltier element. Hybridisation mixtures of 1 ml were prepared containing 10mM Na_2HPO_4 pH 7.0, 0.1mM EDTA and equimolar (1μM) amounts of the different oligonucleotides and their matched and mismatched target DNAs. The T_m values were obtained as the first derivative of the melting curves.

As shown in table 1, the ability of the oxy-LNA modified oligonucleotide (column 2) to differentiate between the complementary DNA target and the targets containing the T, C or G single base mismatches is much better than the corresponding DNA oligonucleotide (column 1). This increased specificity can be improved still further in the case of the T/C mismatch by substituting the oxy-LNA at the mismatch position by the corresponding thio-LNA monomer (column 3). Substituting the two oxy-LNA monomers that flanks the mismatch position with the corresponding thio-LNA (column 4) increases the specificity still further and with effects observed with all three mismatches (T/C, T/T and T/G).

Table 2 shows another example on how thio-LNA can be used to increase the specificity of an oxy-LNA containing oligonucleotide. In this case the centre five monomers in the oligonucleotide are alternating oxy-LNA and DNA monomers. Substituting the oxy-LNA at the mismatch position with the corresponding thio-LNA again increases the specificity of the oligonucleotide most evidently in the case of the T/C and T/T mismatch.

A contiguous strecth of oxy-LNA monomers increases the specificity of an oligonucleotide compared to a strecth of DNA monomers.

The thermostability of different oligonucleotides comprising either DNA monomers or a mix of both DNA and oxy-LNA monomers were determined spectrofotometrically using a spectrophotometer equipped with a thermoregulated Peltier element. Hybridisation mixtures of 1 ml were prepared containing 10mM Na_2HPO_4 pH 7.0, 0.1mM EDTA and equimolar (1μM) amounts of the different oligonucleotides and their matched and mismatched target DNAs. The T_m values were obtained as the first derivative of the melting curves.

As shown in table 3, the ability of the all DNA oligonucleotide (column 1) to discriminate between its matched DNA target and the targets containing T/C, T/T and T/G mismatches can be improved by substituting the centre five DNA monomers with the corresponding oxy-LNA monomers (column 2).

Table 4 shows the result obtained with an oligonucleotide of a different sequence. With the exception of the T/T mismatch, the ability of the oligonucleotide to discriminate between matched and mismatched (T/C and T/G) target DNAs is increased when the centre 5 DNA monomers are substituted by the corresponding oxy-LNA monomers.

Table 1

	5´-gtaggtctctaacga-3´		5´-gtaggT$_O$C$_O$T$_O$C$_O$T$_O$aacga-3´		5´-gtaggT$_O$C$_O$T$_S$C$_O$T$_O$aacga-3´		5´-gtaggT$_O$C$_S$T$_O$C$_S$T$_O$aacga-3´	
	Tm	ΔTm	Tm	ΔTm	Tm	ΔTm	Tm	ΔTm
5´-tcgttag**a**gacctac-3	34.6°C	------	46.8°C	------	48.1°C	------	48.7°C	------
5´-tcgttag**t**gacctac-3	25.6°C	-9.0°C	34.6°C	-12.2°C	35.6°C	-12.5°C	35.4°C	-13.3°C
5´-tcgttag**c**gacctac-3	24.4°C	-10.2°C	32.7°C	-14.1°C	32.8°C	-15.3°C	32.1°C	-16.6°C
5´-tcgttag**g**gacctac-3	28.1°C	-6.5°C	39.1°C	-7.7°C	40.4°C	-7.7°C	40.3°C	-8.4°C

Small letters = DNA monomers, Capital letters with subscript "O" = oxy-LNA, bold capital letters with subscript "S" = thio-LNA . Position of mismatch in target DNA oligonucleotides in bold and underlined.

Table 2

	5´-gtaggT$_O$cT$_O$cT$_O$aacga-3´		5´-gtaggT$_O$cT$_S$cT$_O$aacga-3´	
	Tm	ΔTm	Tm	ΔTm
5´-tcgttagagacctac-3	41.7°C	-------	42.7°C	-------
5´-tcgttagtgacctac-3	33.8°C	-7.9°C	33.3°C	-9.4°C
5´-tcgttagcgacctac-3	31.0°C	-10.7°C	30.0°C	-12.7°C
5´-tcgttagggacctac-3	34.7°C	-7.0°C	35.3°C	-7.4°C

Small letters = DNA monomers, Capital letters with subscript "O" = oxy-LNA, bold capital letters with subscript "S" = thio-LNA . Position of mismatch in target DNA oligonucleotides in bold and underlined.

Table 3

	5′-gtaggtctctaacga-3′		5′-gtagg$T_OC_OT_OC_OT_O$aacga-3′	
	Tm	ΔTm	Tm	ΔTm
5′-tcgttag**a**gacctac-3	34.6°C	------	46.8°C	-------
5′-tcgttag**t**gacctac-3	25.6°C	-9.0°C	34.6°C	-12.2°C
5′-tcgttag**c**gacctac-3	24.4°C	-10.2°C	32.7°C	-14.1°C
5′-tcgttag**g**gacctac-3	28.1°C	-6.5°C	39.1°C	-7.7°C

Small letters = DNA monomers, Capital letters with subscript "O" = oxy-LNA.

Position of mismatch in target DNA oligonucleotides in bold and underlined.

Table 4

	5′-gtaggctttcaacga-3′		5′-gtaggC$_O$T$_O$T$_O$T$_O$C$_O$aacga-3′	
	Tm	ΔTm	Tm	ΔTm
5′-tcgttaga**g**acctac-3	37.1°C	------	47.3°C	-------
5′-tcgttag**t**gacctac-3	28.0°C	-9.1°C	40.0°C	-7.3°C
5′-tcgttagc**g**acctac-3	27.1°C	-10.0°C	35.4°C	-11.9°C
5′-tcgttag**gg**acctac-3	28.8°C	-8.3°C	37.6°C	-9.7°C

Small letters = DNA monomers, Capital letters with subscript "O" = oxy-LNA.

Position of mismatch in target DNA oligonucleotides in bold and underlined.

Claims

1. A method to modulate the ability of an oxy-LNA oligo to discriminate between its complementary nucleic acid target sequences and target sequences that comprise one or more mismatches, by incorporating at least one non-oxy-LNA monomer in the oxy-LNA oligo, wherein the resulting oligo contains at least 50% oxy-LNA and wherein the at least one non-oxy-LNA monomer is either located at or within a distance of no more than three bases from the mismatch position(s).

2. A method according to claim 1, wherein the non-oxy-LNA monomer(s) is/are deoxyribonucleotide(s).

3. A method according to claim 2, wherein the deoxyribonucleotide is modified at the 2'-position in the ribose.

4. A method according to claim 3, wherein the 2'-modification is a hydroxyl, 2'-O-methyl, 2'-fluoro, 2'-trifluoromethyl, 2'-O-(2-methoxyethyl), 2'-O-aminopropyl, 2'-O-dimethyl-amino-oxyethyl, 2'-O-fluoroethyl or 2'-O-propenyl.

5. A method according to claim 3, wherein the modification also involves the 3' position, preferably modifications that links the 2'- and 3'-position in the ribose.

6. A method according to claim 3, wherein the modification also involves the 4' position, preferably modifications that links the 2'- and 4'-position in the ribose.

7. A method according to claim 6, wherein the modification is selected from the group consisting of a 2'-4' link being a $-CH_2-S-$, $-CH_2-NH-$, or $-CH_2-NMe-$ bridge.

8. A method according to any of the claims 2 to 7, wherein the nucleotide has the α-D-ribo, β-D-xylo, or α-L-xylo configuration.

9. A method according to any of the claims 1-8, wherein either all or some of the oxy-LNA monomers or all or some of the non-oxy-LNA monomer(s) or all or some of both the oxy-LNA monomers and non-oxy-LNA monomer(s) contain a 3'- or 5'- modification that results in an internucleoside linkage other than the natural phosphorodiester linkage.

10. A method according to claim 9, wherein the modification is selected from the group consisting of -O-P(O)$_2$-O-, -O-P(O,S)-O-, -O-P(S)$_2$-O-, -NR^H-P(O)$_2$-O-, -O-P(O,NR^H)-O-, -O-PO(R")-O-, -O-PO(CH$_3$)-O-, and -O-PO(NHR^N)-O-, where R^H is selected form hydrogen and C$_{1-4}$-alkyl, and R" is selected from C$_{1-6}$-alkyl and phenyl.

11. A method according to any of the preceding claims, wherein the incorporation of the at least one non-oxy-LNA monomer changes the affinity of the resulting oligo towards its complementary nucleic acid compared to the affinity of the all-oxy-LNA oligo by a ΔT_m of no more than ± 5°C.

12. A method according to claim 11, wherein the affinity is changed by no more than ± 10°C.

13. A method according to any of claims 11 or 12, wherein at least two non-oxy-LNA monomers containing either the same or different nucleobases at the 1'-position and being identical at all other positions are used.

14. A method according to any of claims 11 or 12, wherein at least two non-oxy-LNA monomers containing either the same or different nucleobases at the 1'-position and being non-identical in at least one other position are used.

15. A method according to any of the preceding claims, wherein the oligo has the general formula

$$5'\text{-}X_nY_mX_pY_mX_n\text{-}3'$$

wherein X is oxy-LNA and Y is non-oxy-LNA and n is an integer from 0-50, m is an integer from 0-5 and p is an integer from 0-3.

16. A method according to claim 15, wherein m is an integer from 0-2 and p is an integer from 0-2.

17. A method according to claim 16 wherein m is an integer from 0-1 and p is an integer from 0-1.

18. A method according to any of the preceding claims, wherein the oligonucleotide acts as a substrate for nucleic acid active enzymes.

19. A method according to claim 18, wherein the oligo is used as a substrate for DNA and RNA polymerases.

20. A method according to claim 18, wherein the oligo is used as a substrate for DNA and RNA ligases.

21. The use of an oligo as defined in any of claims 1-17, wherein the oligo is conjugated to a compound selected from proteins, amplicons, enzymes, polysaccharides, antibodies, haptens, and peptides.

22. The use of an oligo as defined in any of claims 1-17 as a therapeutic agent.

23. The use of an oligo as defined in any of claims 1-17 for diagnostic purposes.

24. The use of one or more oligos as defined in any of claims 1-17 in the construction of solid surface onto which oligonucleotides of different sequences are attached.

25. The use according to claim 24, wherein the oligonucleotides are attached in a predetermined pattern.

26. The use of an oligo as defined in any of claims 1-17, wherein the oligo has the function of a ribozyme in the sequence specific cleavage of target nucleic acids.

27. The use of an oligo as defined in any of claims 1-17 in therapy, e.g. as an antisense, antigene or gene activating therapeutic.

28. The use according to claim 27, wherein the oligomer recruits RNase H.

29. The use of complexes of more than one oligo as defined in any of the claims 1-17 in therapy, e.g. as an antisense, antigene or gene activating therapeutic.

30. The use of oligos as defined in any of the claims 1-17 as an aptamer in therapeutic applications.

31. The use of oligos as defined in any of the claims 1-17 in diagnostics, e.g. for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids.

32. The use according to claim 31, wherein the oligo comprises a photochemically active group, a thermochemically active group, a chelating group, a reporter group, or a ligand that facilitates the direct of indirect detection of the oligo or the immobilisation of the oligo onto a solid support.

33. The use according to claim 32, wherein the photochemically active group, the thermochemically active group, the chelating group, the reporter group, or the ligand includes a spacer (K), said spacer comprising a chemically cleavable group.

34. The use of oligos according to any of the claims 1-17 for capture and detection of naturally occurring or synthetic double stranded or single stranded nucleic acids such as RNA or DNA.

35. The use according to claim 34 for purification of naturally occurring double stranded or single stranded nucleic acids such as RNA or DNA.

36. The use of oligos according to any of the claims 1-17 as a probe in *in situ* hybridisation, in Southern hybridisation, Dot blot hybridisation, reverse Dot blot hybridisation, or in Northern hybridisation.

37. The use of oligos according to any of the claims 1-17 in the construction of an affinity pair.

38. The use of oligos according to any of the claims 1-17 as a primer in a nucleic acid sequencing reaction or primer extension reactions.

39. The use of oligos according to any of the claims 1-17 as a primer in a nucleic acid amplification reaction.

40. The use of oligos according to any of the claims 1-17, wherein the oligo is so adapted that the amplification reaction is an essentially linear reaction.

41. The use of oligos according to any of the claims 1-17, wherein the oligo is so adapted that the amplification reaction is an essentially exponential reaction.

42. The use of oligos according to any of the claims 1-17, wherein the nucleic acid amplification reaction results in a double stranded DNA product comprising at least one single stranded end.

43. The use of oligos according to any of the claims 1-17, as an aptamer in molecular diagnostics.

44. The use of oligos according to any of the claims 1-17, as an aptamer in RNA mediated catalytic processes.

45. The use of oligos according to any of the claims 1-17, as an aptamer in specific binding of antibiotics, drugs, amino acids, peptides, structural proteins, protein receptors, protein enzymes, saccharides, polysaccharides, biological cofactors, nucleic acids, or triphosphates.

46. The use of oligos according to any of the claims 1-17, as an aptamer in the separation of enantiomers from racemic mixtures by stereospecific binding.

47. The use of oligos according to any of the claims 1-17 for the labelling of cells.

48. The use of oligos according to claim 47, wherein the label allows the cells to be separated from unlabelled cells.

49. The use of oligos according to any of the claims 1-17 to hybridise to non-protein coding cellular RNAs, such as tRNA, rRNA, snRNA and scRNA, *in vivo* or *in vitro*.

50. The use of oligos according to any of the claims 1-17 in the construction of an oligo containing a fluorophor and a quencher, positioned in such a way that the hybridised state

of the oligomer can be distinguished from the unbound state of the oligomer by an increase in the fluorescent signal from the probe.

51. The use of an oligo according to claim 50 in the construction of Taqman probes or Molecular Beacons.

52. A kit for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids, the kit comprising a reaction body and one or more oligos as defined in any of the claims 1-17.

53. A kit according to claim 52, wherein the oligos are immobilised onto said reactions body.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00549

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12Q 1/68, C12N 15/11, C07H 21/00 // A61K 48/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12Q, C12N, C07H, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9914226 A2 (EXIQON A/S), 25 March 1999 (25.03.99), page 57, lines 21-27; page 61, line 15 - page 62, line 2	1-53
A	EP 0415901 A2 (MONSANTO COMPANY), 6 March 1991 (06.03.91)	1-53

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 January 2001	26. 02. 2001
Name and mailing address of the ISA/ European Patent Office Facsimile No.	Authorized officer PATRICK ANDERSSON/EÖ Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
pct/dk00/00549

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 22, 27-30 completely, 23, 31-35, 43 partially
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK00/00549

Claims 22, 27-30 completely, 23, 31-35, 43 partially relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

04/12/00

International application No.
PCT/DK 00/00549

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9914226 A2	25/03/99	AU 9063398 A	05/04/99
		EP 1015469 A	05/07/00
		US 5996170 A	07/12/99
EP 0415901 A2	06/03/91	CA 2024182 A	01/03/91
		JP 1967673 C	18/09/95
		JP 3098599 A	24/04/91
		JP 6102676 B	14/12/94
		US 5134066 A	28/07/92
		US 5359053 A	25/10/94